Preservation of Reverse Osmosis Membranes From Microbial Attack

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SUMMARY

Cellulose acetate reverse osmosis membranes were inoculated with a mixed bacterial culture, *Cryptococcus albidus* yeast and a *Penicillium* sp. mold, respectively, to determine if any of these common maple sap organisms would be capable of destroying the membranes. No destructive effect was observed but severe sliming of the membrane by the mixed bacterial culture was noted.

When the reverse osmosis unit was operated daily for concentration of a 2.5% sucrose solution, the bacterial population of the feed solution was held to a minimum by the use of in-line germicidal ultraviolet lights. Bacterial populations of both concentrate and permeate effluent streams decreased during periods of operation. However, increased bacterial counts in effluent samples taken at the start of operations indicated that bacterial growth took place in both concentrate and permeate sectors of the pressure vessels when the reverse osmosis unit was idle. It was found that sanitization of the reverse osmosis unit with a sodium hypochlorite sanitizer containing 50 ppm available Cl_2 acidified to pH 4.5–4.7 with glacial acetic acid maintained the unit in good sanitary condition for idle periods as long as three days.

INTRODUCTION

One of the more successful applications of the reverse osmosis process in the food industry is for the partial concentration of maple sap as an intermediate step in the production of maple sirup (Willits et al., 1967 and Underwood et al., 1969). At present, maple sirup is produced by boiling maple sap (1.0°–4.0° Brix) in an open pan atmospheric evaporator, until the desired sirup density, 66° Brix, is attained.

The reverse osmosis process is ideally suited for the partial concentration of maple sap. Seventy-five percent of the water can be removed by this process at 1/25 the energy cost of the oil fuel used in the conventional boiling procedure (Underwood et al., 1969). Since removal of 75% of the water raises the Brix to only 10°–12°, the opposing osmotic pressure is not excessive. The concentration is done at room temperature so that there is a minimum of heat damage to the dilute sap and the final stage of sap concentration, raising the Brix from 10°–12° to 66°, can be economically done by boiling in conventional open pan evaporators. This provides the heat treatment needed to produce the maple color and flavor.

However, the most important potential of this process is that it fits logically into the pattern of the central plant concept for maple sap evaporation. Small reverse osmosis plants could be located within a 100-mile radius of the central plant to reduce the sap to 1/4 its original volume so that it can be more economically hauled to the central plant. Whether the reverse osmosis plant is used in the field or as an integral part of the central concentration plant, it is of utmost importance that the reverse osmosis membranes be kept undamaged by microbial action while in use and during periods of shut-down, as well as during the 10–11 months between sap seasons.

It was recognized that microorganisms could cause damage to the modified cellulose acetate membranes of the reverse osmosis modules or form slime deposits on the membranes capable of causing a marked decrease in the flux rate of water through the membranes. Thus, it was necessary to devise means of reducing the microbial population in the sap to the lowest possible level before it was supplied to the reverse osmosis unit. Methods also had to be developed for control of microbial growth in reverse osmosis apparatus during periods of idleness.

Since the production of maple sap is intermittent and unpredictable as to time and volume, the supplies of sap to be concentrated by reverse osmosis are likewise intermittent, resulting in the equipment being idle for periods of two days to a week during the maple season. In addition, the sap season never exceeds two months in duration so that the equipment is also idle for a minimum of 10 months per year.

The problem of maintaining the reverse osmosis equipment in good sanitary condition during idle periods was complicated by the equipment design. The reverse osmosis equipment operates as a closed system which cannot be completely drained or readily disassembled for sanitization. Moreover, the spirally wound membrane modules used in this study were manufactured as permanently sealed units. Because of these design factors, the EUROC (Eastern Utilization Reverse Osmosis Concentrator) unit could only be sanitized using in-place cleaning techniques. This was further complicated by the fact that maple sirup is a food product, and because of this, a sanitizer was required which could be washed from the reverse osmosis unit without leaving residues unacceptable to food regulatory laws or capable of creating "off" flavors in the sirup. Also, an acidic sanitizer was needed, because contact with alkaline liquids has an adverse effect on the semi-

permeable membranes of the reverse osmosis modules (Larson, 1967).

This paper presents the results of initial studies conducted to develop procedures for the prevention of microbial damage to reverse osmosis membranes and for maintenance of sanitary conditions in reverse osmosis equipment.

MATERIALS AND METHODS

EUROC reverse osmosis unit. 1. A reverse osmosis unit (EUROC), capable of processing sap at feed rates up to 12 gpm and pressures up to 700 psig was designed and constructed at this laboratory (Moore et al., 1968).

2. The reverse osmosis membranes were spirally wound modules (ROGA modules) 4 in. in diameter × 12 in. long, containing approximately 10 sq ft of membrane and were obtained from Gulf General Atomics.

Ultraviolet irradiation. 1. Two Aquafine model SP-2 ultraviolet irradiation units, each consisting of two 36 in. germicidal lamps, were mounted so that the sap feed passed over the tubular lamps in a ½ in. concentric layer. The two units were assembled in series in the feed lines down-stream from the filters and on the suction side of the pump.

2. Two 36 in. germicidal lamps with reflectors were mounted above the sap feed supply tank so as to irradiate the entire surface of the sap contained in the tank.

Sanitizer solution for EUROC system. Sanitization of the EUROC system was carried out using a commercial alkaline hypochlorite solution diluted with water to provide 50 ppm available chlorine and acidified to pH 4.5 to 4.7 with glacial acetic acid.

Microorganisms. 1. Mixed bacterial culture. The mixed culture contained Pseudomonas, Bacillus, Leuconostoc spp. and other natural sap contaminants isolated from commercially produced sap. The mixed culture was maintained on tryptone glucose extract agar (Difco) slants at 30°C and was transferred at 48 hr intervals.

2. *Cryptococcus albidus* yeast culture. The yeast culture was isolated from naturally contaminated maple sap. It was maintained on Wort agar (Difco) slants at 20°C and was transferred at 5 day intervals.

3. *Penicillium* sp. mold. The mold culture was isolated from naturally contaminated maple sap. It was maintained on acidified potato dextrose agar (Difco) slants at 20°C and was transferred at 5 day intervals.

Culture media. 1. Tryptone glucose extract agar (Difco) was used for all bacterial counts and bacterial stock cultures.

2. Wort agar (Difco) was used for all yeast counts and yeast stock cultures.

3. Acidified potato dextrose agar (Difco) was used for all mold counts and mold stock cultures.

Action of microorganisms on reverse osmosis membranes. A spirally wound reverse osmosis module consisting of membrane, backing material and separators was opened and sections of cellulose acetate membrane 2 in. × 1 in. were cut from the module using aseptic precautions. Three petri dishes were prepared with 15 ml volumes of T.G.E., Wort and acidified potato dextrose agar, respectively and the agar allowed to solidify. One of the sections of membrane was laid flat on the agar surface of each of the prepared dishes. The membranes were then streaked with an inoculum.

The mixed bacterial culture was used to inoculate the membrane on the T.G.E. substrate; the Cryptococcus albidus for the membrane on the Wort agar; and the Penicillium sp. mold for the membrane on the acidified potato dextrose agar. Another section of the membrane was immersed in maple sap which had been inoculated with the mixed bacterial culture.

The membranes inoculated with the mixed bacterial culture were incubated at 30°C for 48 hr, and those inoculated with the yeast and mold cultures were incubated at 20°C for 5 days. Following the initial incubations, all cultures were held at room temperature and examined daily for evidence of destructive microbial action on the membranes for a period of one month at which time the membranes were rinsed clean, air dried and examined for visible evidence of destructive action.

Microbiological studies with the EUROC unit. A flow diagram of the EUROC reverse osmosis maple sap concentration process is shown in Figure 1. The initial studies to determine sanitation procedures were made using a 2.5° Brix sucrose-water solution. The microbial population of the sugar feed solution was controlled by continually irradiating the feed solution with overhead ultraviolet lamps and continuously renewing the surface exposed to the actinic rays by stirring. This sugar solution was passed first through a Cuno P 110 cartridge filter and then through the in-line irradiation units before being pumped into the pressure vessels of the EUROC. The two effluent streams from the concentrator, the permeate and the concentrate, were recycled to the feed tank.

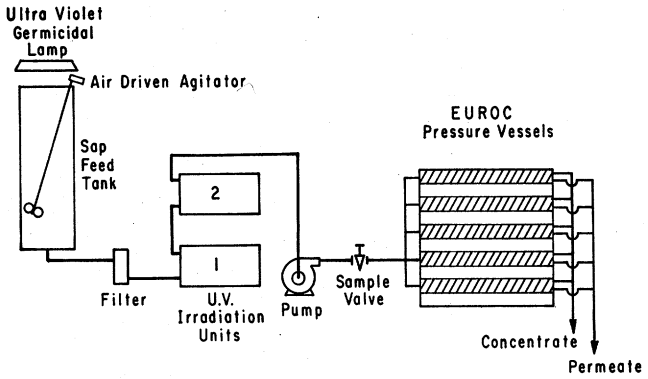

Fig. 1. Flow diagram of EUROC reverse osmosis maple sap concentration process.

When the EUROC was operated on successive days, the unit was flushed out at the end of each day's operation by pumping 200 gallons of irradiated tap water through the system. This water, having passed through the Aquafine units at a constant rate of 6 gpm, was essentially sterile (Robe et al., 1967). The EUROC was sanitized with the hypochlorite, if the unit was to stand idle for more than one day. This was done by pumping 100 gallons of sodium hypochlorite solution containing 50 ppm available Cl_2 and acidified to pH 4.5 to 4.7 through the system at a rate of 6 gpm. The sanitization was followed by a rinse of 200 gallons of tap water. Both the sanitizing solution and the rinse tap water were irradiated by passage through the Aquefine units.

Sampling and plating. During the the initial studies in which the 2.5° Brix sucrose-water solution was recirculated through the EUROC unit, samples for bacterial, yeast and mold counts were taken aseptically from the sample valve in the sap feed line and from the concentrate and permeate discharge lines at the start and finish

of each day's operation.

The samples were diluted and plated according to conventional methods. All dilutions were made with phosphate buffered distilled water (Am. Public Health Assoc., 1960). All media were sterilized by autoclaving at 121°C for 15 min. Bacterial cultures were incubated at 30°C for 48 hr. Yeast and mold cultures were incubated at 20°C for 5 days. All plate counts were made using a Quebec colony counter.

RESULTS AND DISCUSSION

Laboratory Studies. Preliminary studies were made to determine the effects of the growth of microorganisms associated with maple sap on reverse osmosis membranes. As previously described, sections of membrane were placed on the solidified surfaces of appropriate agar substrates, inoculated with the test cultures and incubated. The mixed bacteria culture producted a sparse, mucoid growth which turned brown in color and dried on the surface of the modified cellulose acetate membrane within a week following the initial incubation. The Penicillium sp. mold which normally produces a luxuriant gray-green mycelial mass, produced only an atypical, sparse, starved, white mycelial growth on the membrane surface. Inoculation of the membrane surface with *Cryptococcus albidus* yeast resulted in the drying of the original inoculum on the membrane surface.

One month after the initial incubation, the membranes were rinsed clean, air-dried and examined with a stereomicroscope (10× magnification using both direct and transmitted light) for visible evidence of destructive action by the test microorganisms. With the exception of a slight yellowish discoloration noted on the membrane which had been inoculated with the mold culture, no evidence of microbial activity was observed. This indicated that growth of these common sap contaminants would not result in destruction of the cellulose acetate membranes, but the sparse bacterial and mold growths noted suggested that slime layers could develop on the membrane surface which might adversely affect the rate of permeation (flux rate) of water through the membrane.

The problem of slime development on membrane surfaces was further emphasized by a study in which a section of modified cellulose acetate membrane was submerged in maple sap, inoculated with the mixed culture of sap bacteria, and incubated at room temperature for two weeks. A heavy accumulation of slime developed on the membrane surface within three days following inoculation. After one month's incubation, the membrane was removed, rinsed clean and air dried. The membrane was examined with the stereomicroscope, and there was no visible evidence of damage to the membrane.

These studies did not simulate conditions encountered in the operation of the EUROC. They were conducted as static, aerobic incubations at atmospheric pressure with rigidly controlled incubation temperatures during the initial portion of the studies. During the subsequent holding periods at room temperature, the temperature fluctuations were less than ±5°F from 72°F. Nevertheless these studies indicated that, although the cellulose acetate membranes were not decomposed by these microorganisms commonly associated with maple sap, overgrowths of bacteria or molds could occur which might impair the flux rate of water through the membranes. The studies further showed that the microbial growths produced by common sap contaminants could be readily washed from the membranes.

In operation, the membranes in the spirally wound modules used in the pressure tubes of the EUROC are subjected to pressures varying from 350 to 700 psi; temperatures ranging from 40° to 70°F; and conditions of aeration ranging from an aerobic environment during operation to an environment having reduced oxygen levels during periods of idleness. These unstable environmental conditions are not conducive to rapid microbial growth.

Several modules were opened after 4 months of discontinuous operation during which time sucrose solutions and maple sap had been concentrated. Examination of the membranes from the opened modules gave no evidence of appreciable bacterial, yeast or mold growth. This confirmed the preceding observations that the cellulose acetate membrane does not provide a suitable growth medium and that the destruction of the membrane by microorganisms associated with maple sap is a minor consideration. It was also apparent that there had been no deposition of microbial slime on the membrane surface during the four-month period in which the EUROC had been in intermittent operation.

A study was made to determine the effects of intermittent operation on microbial populations of the EUROC vessels and to determine the effects of sanitization of the EUROC system prior to short periods of idleness on the growth of these organisms. Since maple sap was not available for this study, a 2.5° Brix sucrose solution was used in place of fresh sap. The sucrose solution was recycled through the EUROC system with both concentrate and permeate effluent streams being returned to the feed tank.

The microbial population of the sugar solution used as feed to the EUROC was controlled by irradiating the surface of the solution as it was held in the feed tank with overhead ultraviolet lamps. The feed solution was stirred continuously to renew the surface layer exposed to the ultraviolet rays and thereby obtain the maximum germicidal effect. Further control of the microbial population was achieved by passing the feed solution through the in-line irradiation units. This maintained the bacterial population of the feed solution below 70 cells/ml during the study. The sugar solution was pumped through the EUROC pressure vessels at 6 gpm under 600 psig pressure. Samples for bacterial, yeast and mold counts were taken aseptically from the feed line sample valve and from the concentrate and permeate streams at the start and end of each day's operation.

The results of this study are shown in Figures 2 and 3. Only bacterial populations were plotted, since the yeast and mold counts of all samples were <50 organisms/ml. Figures 2 and 3 show data from two 8-day periods during which the EUROC was operated intermittently. In each operational period, the EUROC was used to concentrate the sucrose solution for 2 to 6 hr per day on 3 successive days, stood idle for 3 days, and then was operated for 2 to 6 hr per day on 2 successive days. At the end of operation on the first, second and seventh days, the EUROC was rinsed with 200 gallons of water irradiated by passage through the in-line ultraviolet units.

In the first operating sequence (Fig. 2), the EUROC was sanitized at the end of the third day of operation by pumping 100 gallons of acidified hypochlorite solution through it at a flow rate of 6 gpm. The residual santizer solution was rinsed from the unit with 200 gallons of irradiated water. In the second operating period (Fig. 3), the EUROC was not sanitized at the end of the third day of operation but was rinsed with 200 gallons of irradiated water.

Figures 2 and 3 show that the bacterial populations of the concentrate and permeate streams followed a definite trend during the study. The bac-

terial concentrations in both concentrate and permeate streams showed steady decreases from the start to the end of each day's operation, reflecting the dilution effect brought about by the low bacterial population in the irradiated feed solution.

Even when the EUROC was operated for only 2 hr (Fig. 6, second day), bacterial counts declined from 2.5×10^5 to 1.5×10^5 cells/ml in the concentrate effluent and from 1.4×10^4 to 1.2×10^4 cells/ml in the permeate. During the short idle periods between the daily operations, the bacterial populations in the pressure vessels recovered as evidenced by the higher populations of concentrate and permeate samples taken at the start of each day's operation.

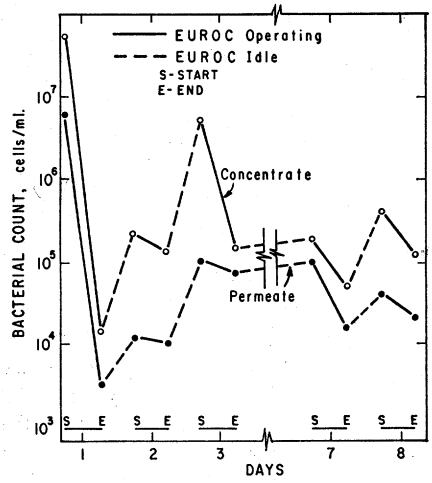

Fig. 2. Bacterial counts of concentrate and permeate effluents at start and end of daily EUROC operation. EUROC sanitized at end of third day's operation prior to a 3-day idle period. S = start of day's operation, E = end of day's operation.

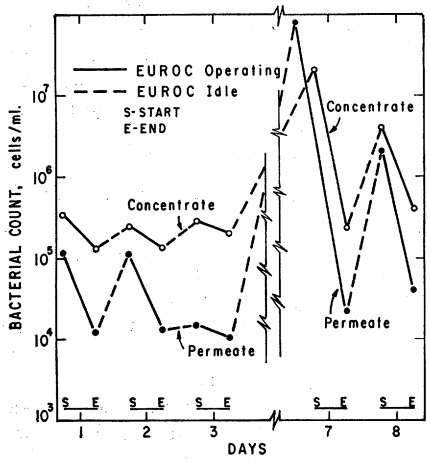

Fig. 3. Bacterial counts of concentrate and permeate effluents at start and end of daily EUROC operation. EUROC not sanitized at end of third day's operation prior to 3-day idle period. S = start of day's operation, E = end of day's operation.

In the first period of operation (Fig. 2), the EUROC was sanitized at the end of the third day. The unit stood idle for 3 days with ambient temperatures ranging from 65°–70°F. When the unit was again put in operation on the seventh day, samples taken at the start showed bacterial populations in the concentrate and permeate streams of 2.0×10^5 cells/ml and 1.1×10^5 cells/ml, respectively. This indicated that even after sanitization, the residual bacteria in the pressure vessels multiplied at ambient temperature during the 3-day idle period to population levels comparable to those found at the end of the third day's operation.

Failure to sanitize the EUROC prior to the 3-day idle period resulted in a massive increase in the bacterial populations of both the permeate and concentrate sectors of the pressure vessels as shown in Figure 3 in comparison to the data shown in Figure 2. At the start of operation on the seventh day, both effluent streams contained bacterial populations in excess of 2.0×10^7 cells/ml. This exceeded by 100-fold the population levels found in identical samples taken after the EUROC had been sanitized (Fig. 2).

It also was apparent that failure to sanitize the EUROC prior to the 3-day idle period permitted the development of contamination foci in the pressure vessels because even though the bacterial populations in the permeate and concentrate decreased to 2.5×10^4 and 2.7×10^5 cells/ml, respectively, by the end of the seventh day, the short idle period before start-up on the eighth day resulted in more rapid population increases than seen in Figure 2 (to more than 2.0×10^6 cells/ml in both effluents). This indicated that the EUROC required sanitization before periods of idleness as short as 3 days and suggested that periodic sanitization would be required to maintain the unit in good sanitary condition during prolonged periods of idleness.

Further sanitation studies will be conducted under commercial plant conditions in which fresh maple sap will be partially concentrated by this process prior to conversion to sirup by atmospheric evaporation.

CONCLUSIONS

1. Streak inoculation of sections of reverse osmosis membrane with respective cultures of mixed sap bacteria, Cryptococcus albidus yeast, and a Penicillium sp. mold produced neither significant growth on nor visible damage to the membranes. However, cellulose actetate membranes immersed in maple sap which had been inoculated with the mixed culture of sap bacteria become heavily coated with microbial slime during two weeks' incubation at room temperature.

2. The use of ultraviolet germicidal lamps in the maple sap feed supply line effectively reduced the bacterial population of the sap supplied to the membrane modules.

3. The bacterial population in the pressure vessels of the EUROC increased when the unit stood idle, but in the course of these studies, the increase in population did not appear to damage the modules nor have any effect on subsequently obtained simulated sap concentrates.

4. The EUROC system can be maintained in good sanitary condition during idle periods as long as 72 hr by in-place cleaning with a sodium hypochlorite solution containing 50 ppm available chlorine acidified to pH 4.5 to 4.7 with glacial acetic acid followed by rinsing with tap water irradiated by the in-line germicidal lamps.

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